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Glucose transporter gene expression in rat conceptus during high glucose culture

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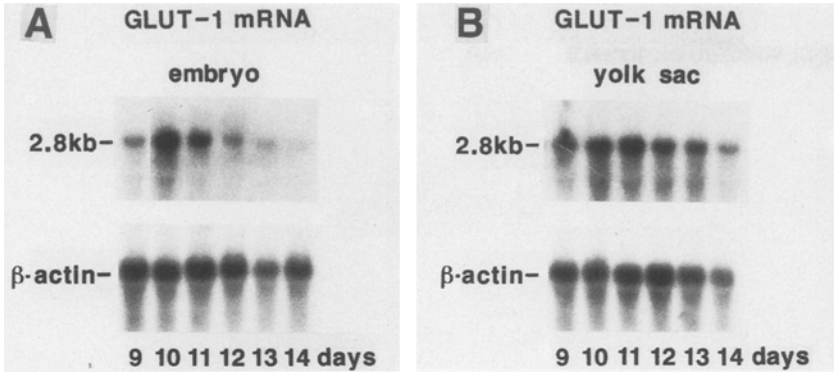
Summary. We investigated the expression of glucose transporter genes and protein in embryo and yolk sac during organogenesis and the regulation of glucose transporters during culture in hyperglycaemic media. Erythrocyte-type glucose transporter (GLUT 1) and brain-type glucose transporter (GLUT 3) mRNA were expressed in embryo and yolk sac. The expression of GLUT-1 and GLUT-3 mRNA was abundant on day 9–11 and day 9–10 in the embryo, respectively, and day 9–14 and day 10–11 in the yolk sac, respectively. The levels of GLUT-1 protein in the embryo increased in parallel with the expression of GLUT-1 mRNA during the corresponding period. Immunohistochemical staining of GLUT-1 protein was found principally in the neuroepithelial cells surrounding the neural tube in the embryo on day 10 and appeared in the microvessels surrounding the neural tube after day 12.

To test whether the expression of glucose transporter genes and protein was suppressed during hyperglycaemia, conceptuses were cultured in high glucose medium. The abundant expression of GLUT-1 protein was not decreased during culture in high glucose media for 24 h (day 9–10) and was only down-regulated by prolonged exposure to this media for 48 h (day 9–11). We have demonstrated the predominant expression of the high affinity glucose transporter (GLUT 1 and GLUT 3) genes and (GLUT 1) protein in embryo during the early period of organogenesis. The persistently abundant expression of glucose transporter during the critical period of neural tube formation (day 9–10) even in the presence of hyperglycaemia may explain one of the mechanisms of increased glucose flux into the neuroepithelium, which may lead to neural tube defects.

Key words: Glucose transporter, embryogenesis, hyperglycaemia, rat embryo culture.

The transport of glucose into the cell is catalysed by glucose transporters, for which the cDNAs encoding five facilitative glucose transporter isoforms (the erythrocyte/GLUT 1, liver/GLUT 2, brain/GLUT 3, muscle-fat/GLUT 4 and small intestine/GLUT 5) have been isolated and characterized [1–9]. GLUT 1 and GLUT 3 are expressed at various levels in many human tissues and are most abundant in the brain. These two glucose transporters have a low K_m for glucose. GLUT 2 is expressed in the liver and pancreatic beta cell. This isoform has a high K_m and may mediate the bidirectional transfer of glucose across the plasma membrane of the liver and the glucose-sensing mechanism of pancreatic beta cell. GLUT 4 is expressed in skeletal muscle, cardiac muscle and fat and is responsible for the insulin-stimulated uptake of glucose.

The postimplantation conceptus in the rat consists of embryo and extraembryonic membrane such as yolk sac, amnion and allantois. The yolk sac is initially the site for the transfer of glucose between mother and embryo before the chorioallantoic circulation is established. During the early period of organogenesis (day 9–10), the rat conceptus is totally dependent on anaerobic glycolysis which is characterized by a high rate of glucose utilization and conversion to lactic acid, with little activity of oxidative metabolism through the Krebs cycle [10, 11]. These two days of the embryo correspond to the main period of neurulation. During the latter part of gestational days 11 and 12 when the tricarboxylic acid cycle begins to function, the rate of anaerobic glycolysis declines [10, 11]. Thus, the glucose requirement dramatically changes during the development of the rat conceptus. The relationship between derangement of energy metabolism and abnormal development of the postimplantation conceptus has been reported [12–27]. When embryos were cultured in the glucose-deficient or mannose-supplemented media, malformations such as neural tube defects in association with reduction of glycolytic flux, were observed suggesting that interruption of glycolytic flux before the maturation of the Krebs cycle (day 9–10) might result in general growth retardation and neural tube defects [12–16].



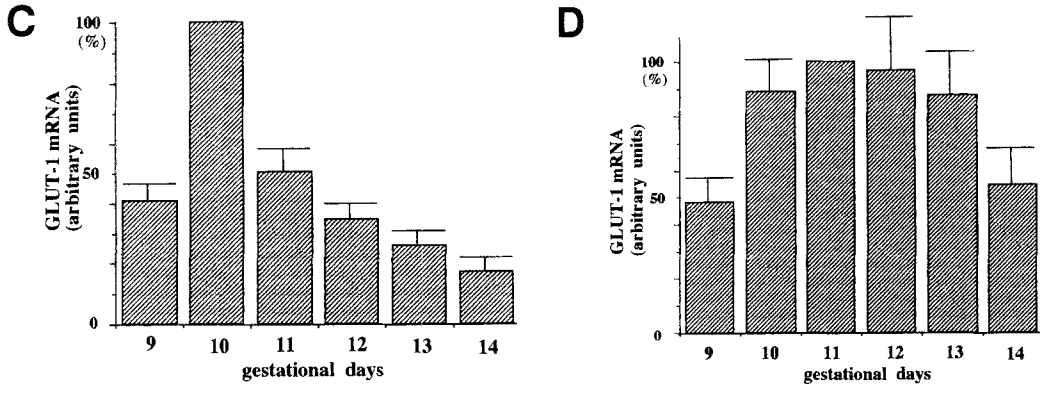
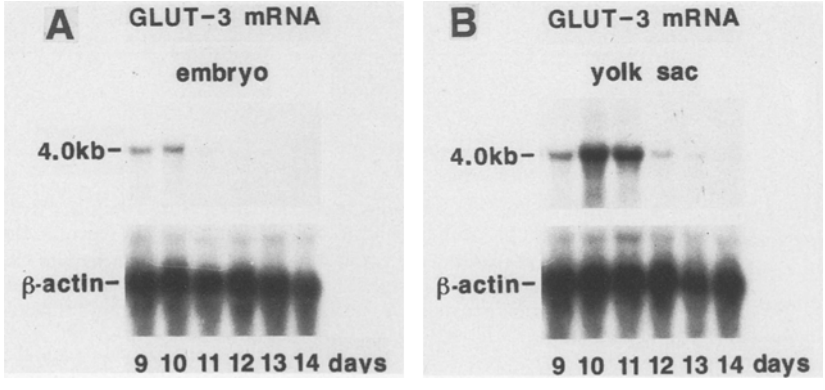


Fig. 1 A–D. Representative Northern blot (**A**, **B**) and quantitation (**C**, **D**) of GLUT-1 mRNA from the embryo and yolk sac from day 9–14 of gestation. Rat conceptuses were collected at daily intervals. Total RNA was extracted from the embryo and yolk sac on each gestational day. RNA (30 μg) was subjected to electrophoresis, and transferred to nylon paper. The nylon filters were hybridized with the denatured cDNA probe of GLUT-1 labelled with ^{32}P-dCTP and the autoradiograms were quantified using scanning densitometry. The optical density values were expressed in arbitrary units of absorbance relative to the value of the stages showing the maximal level of GLUT-1 mRNA. Each bar is the mean ± SEM of five independent determinations



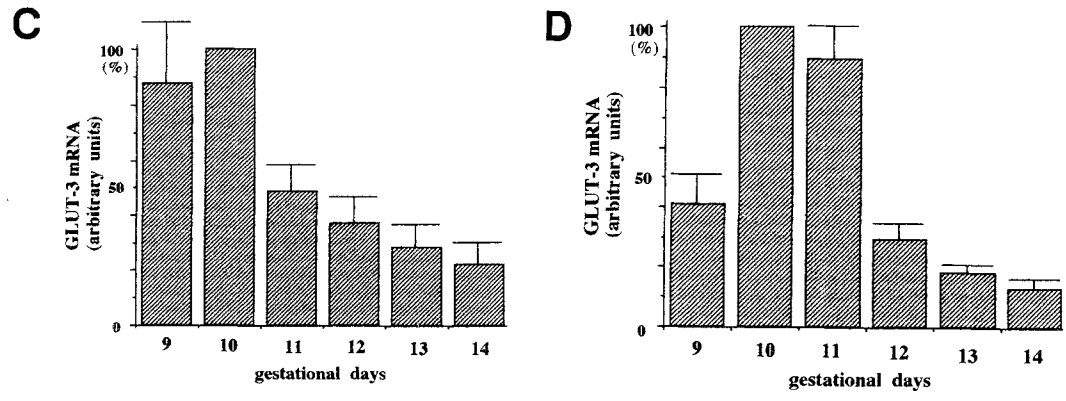


Fig. 2 A–D. Representative Northern blot (**A**, **B**) and quantitation (**C**, **D**) of GLUT-3 mRNA from the embryo and yolk sac from gestational day 9–14. Northern blot analysis was performed as described in Figure 1. The optical density values were expressed in arbitrary units of absorbance relative to the value of the stages showing the maximal level of GLUT-3 mRNA. Each bar is mean ± SEM of three independent determinations

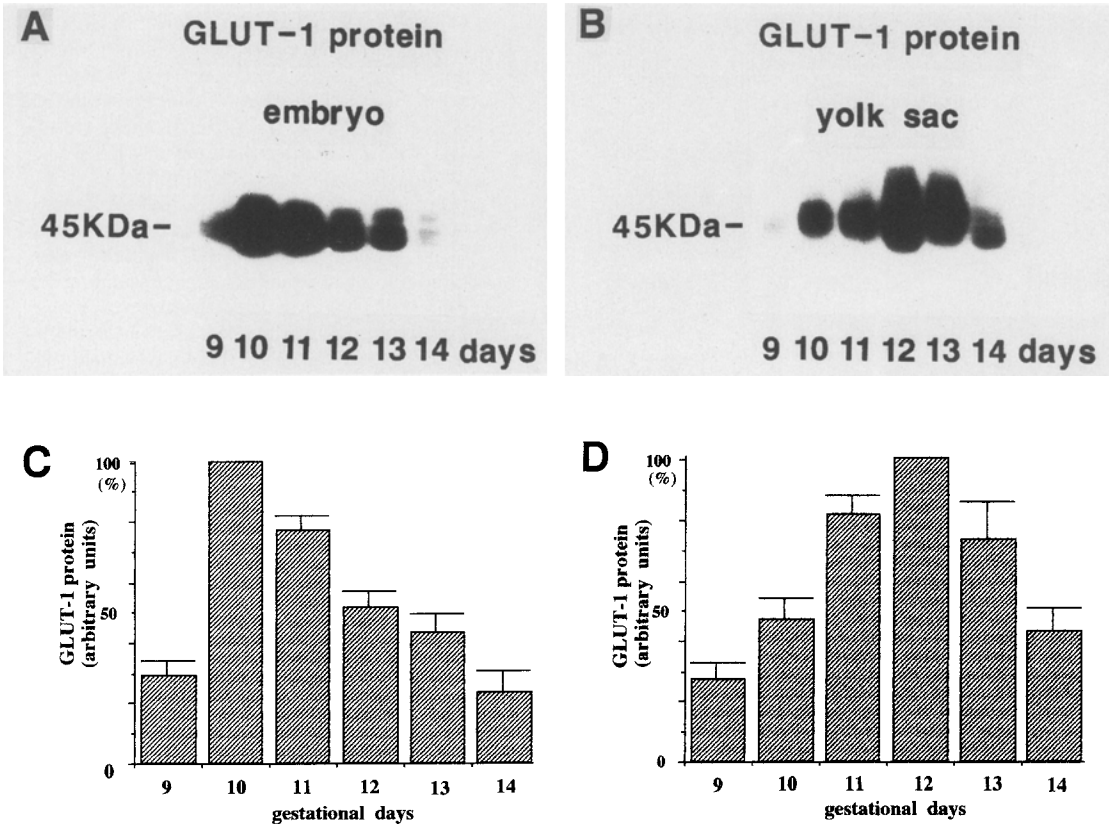


Fig. 3 A–D. Representative Western blot (**A**, **B**) and quantitation (**C**, **D**) of GLUT-1 protein from the embryo and yolk sac from day 9–14. Rat conceptuses were collected as described in Figure 1. Protein (50 µg) in the embryo and yolk sac was resolved on SDS-PAGE, transferred to nitrocellulose paper, incubated with GLUT-1 antiserum (1:2500) followed by ^{125}I-labelled protein A and subjected to autoradiography. The optical density values were expressed in arbitrary units of absorbance relative to the value of the stages showing the maximal level of GLUT-1 protein. Each bar is mean ± SEM of four independent determinations

Addition of glucose to culture media (day 9–10), has been shown to cause disturbed development and malformation [17–21]. These effects might be mediated by an increased glucose flux, resulting in myo-inositol depletion or generation of free oxygen radicals in the embryos at a critical stage of organogenesis [22–27].

As for the regulation of glucose transporters, an increased expression of GLUT 1 has been shown to be induced by factors that promote cell growth and division such as oncogenes, growth factors and tumour promotors [28, 29]. It has also been reported that ambient glucose can affect both glucose transport activity and levels of the GLUT gene, and protein; increased activity of glucose transport or increased expression of GLUT, or both, may occur in the absence of glucose [30–37], while decreased activity or decreased expression, or both, are induced by the presence of high glucose [36, 38–43].

We have investigated the expression of glucose transporter genes and proteins in embryo and yolk sac during the period of organogenesis. The regulation of the glucose transporter (GLUT 1, GLUT 3, GLUT 4) genes and (GLUT 1) protein was investigated in conceptuses to evaluate one of the mechanisms for excess accumulation of glucose in the embryo during high glucose exposure, which may lead to neural tube defects.

Materials and methods

Virgin female Wistar rats were mated and pregnancy was timed from the midnight preceding the morning when sperm was present in the vaginal smear. On day 9–14 of gestation, mothers were killed by cervical dislocation and conceptuses were collected at daily intervals for Northern blot analysis, Western blot analysis and immunohistochemistry.

Embryo culture

Embryo culture techniques were performed using the methods of New [44]. On day 9 of gestation, conceptuses were excised, floated in petri dishes containing Hanks' balanced salt solution and freed of decidua. Reichert's membrane was opened and intact embryo units (embryos together with their visceral yolk sac, amnion and ectoplacental cone) were explanted into culture media. Sterile culture media consisted of the immediately centrifuged, heat-inactivated serum from normal female rats diluted 3:1 with isotonic saline (0.85% NaCl) to obtain a final glucose concentration of 6.6 mmol/l as control medium. The latter was isosmotically replaced by 5% D-glucose in saline to obtain a high glucose medium containing additional 33.3, 66.6 mmol/l glucose concentrations. Each culture tube containing six embryo units in 6 ml medium was incubated at 37°C with rotation in a temperature-controlled incubator. Embryo units were transferred to new culture vessels after 24 h and resuspended in 6 ml of fresh culture medium for the second 24-h period. The embryo units were removed from the culture vessels at the end of 48 h on day 11 of development.

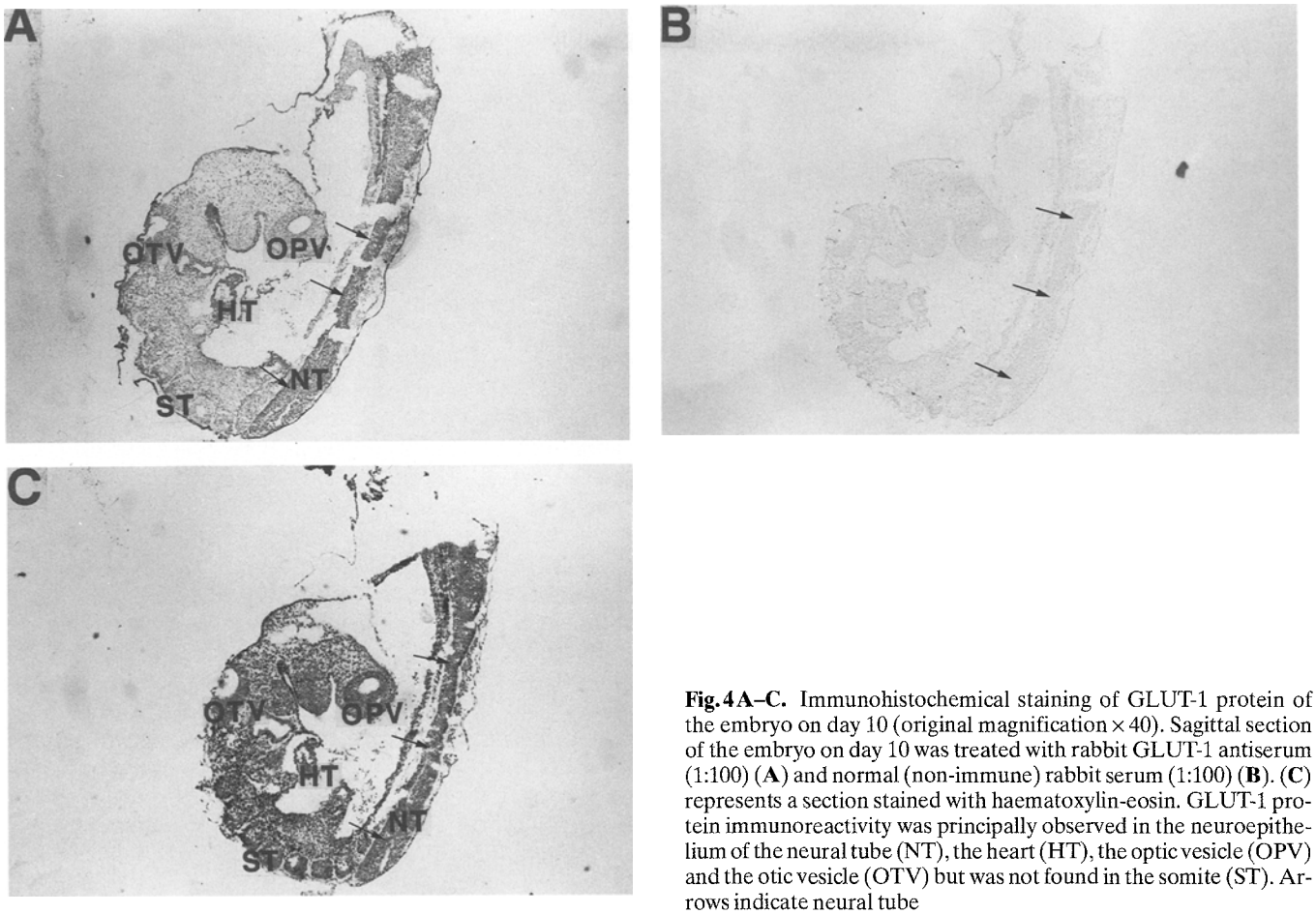


Fig. 4A–C. Immunohistochemical staining of GLUT-1 protein of the embryo on day 10 (original magnification × 40). Sagittal section of the embryo on day 10 was treated with rabbit GLUT-1 antiserum (1:100) (**A**) and normal (non-immune) rabbit serum (1:100) (**B**). (**C**) represents a section stained with haematoxylin-eosin. GLUT-1 protein immunoreactivity was principally observed in the neuroepithelium of the neural tube (NT), the heart (HT), the optic vesicle (OPV) and the otic vesicle (OTV) but was not found in the somite (ST). Arrows indicate neural tube

Northern blot analysis

Embryos were quickly separated from the yolk sac and amnion under a dissecting microscope, immediately frozen and stored in liquid nitrogen to ensure the preservation of RNA. Embryos and yolk sacs were homogenized and total cellular RNA was extracted by the acid guanidinium thiocyanate-phenol-chloroform method [45]. Total RNA (30 μg) was denatured, electrophoresed on 1% formaldehyde agarose gels, blotted, transferred to a nylon membrane (Hybond N; Amersham, Arlington Heights, IL., USA) and then fixed under ultraviolet light. The GLUT-1 probe, a 2.4 kilobase (kb) pair fragment of the HEpG2 cDNA which included the region downstream from the internal *Bam* HI site [3], GLUT-3 probe, 562 base pair (bp) fragment of mouse GLUT-3 cDNA [7] and GLUT-4 probe, 795 bp fragment of polymerase chain reaction (PCR)-amplified rat muscle cDNA [8], were labelled with [α-^{32}P] dCTP (Amersham) by a modified random priming technique and separated from the unincorporated dCTP by gel filtration chromatography. These probes were hybridized to RNA immobilized on the nylon membrane in a solution containing 50% formamide, 5 × Denhardt's solution, 1% sodium dodecyl sulphate (SDS) and 5 × SSPE (1 × SSPE = 150 mmol/l NaCl, 10 mmol/l NaH_2PO_4, 1 mmol/l EDTA, pH 7.4) at 42°C for 24 h. The membrane was washed twice in 2 × SSC (1 × SSC = 150 mmol/l NaCl, 15 mmol/l sodium citrate, pH 7.4), 0.1% SDS at room temperature and once in 1 × SSC, 0.1% SDS for 20 min at 55°C. The membrane was exposed to the Kodak X OMAT AR film at −70°C for 3 days. After stripping of the glucose transporter probe, the papers were then rehybridized with β-actin cDNA probe labelled with [α-^{32}P] dCTP. The autoradiographic bands obtained by Northern blotting were then quantified by scanning densitometry.

Western blot analysis

Embryo and yolk sac were separately thawed in a homogenizing buffer consisting of 20 mmol/l HEPES, 1 mmol/l EDTA, 250 mmol/l sucrose, 1 mmol/l phenylmethylsulphonyl fluoride, 10 mmol/l iodoacetoamide, and 1% Triton X-100 with a dounce homogenizer. Homogenates were centrifuged at 12,000 rev/min at 4°C for 10 min, and the protein content determined by the Bio-Rad protein dye assay with bovine plasma gamma globulin as standard. Total protein (50 μg) was resolved by SDS-polyacrylamide gel electrophoresis (PAGE) using a 10% gel, electrically transferred to nitrocellulose paper. The blots were blocked with mild shaking in phosphate-buffered saline (PBS) with 5% powdered milk at 4°C overnight. They were then incubated with a 1:2500 dilution of a GLUT-1 antiserum raised against the COOH-terminal of rat brain glucose transporter [1] in PBS containing 1% powdered milk for 60 min at 37°C and incubated with ^{125}I-labelled protein A (Amersham). The filters were washed, air dried, and exposed to Kodak X OMAT AR film at −70°C for 3 days. Bands on the autoradiograms were quantified by scanning densitometry.

Immunohistochemistry

After the quick separation of embryo from membrane fraction under the dissecting microscope, the embryo was immersed and fixed in 4% paraformaldehyde, and immersed in 20% sucrose and embedded in O.C.T. compound (Tissue Teck, Los Angeles, CA, USA). The frozen sections were cut at 4–6 μm on a Bright cryostat. After pretreatment with 0.3% H_2O_2 and normal rabbit sera, 1-h incubation at room temperature with GLUT-1 antiserum (1:100 dilu-

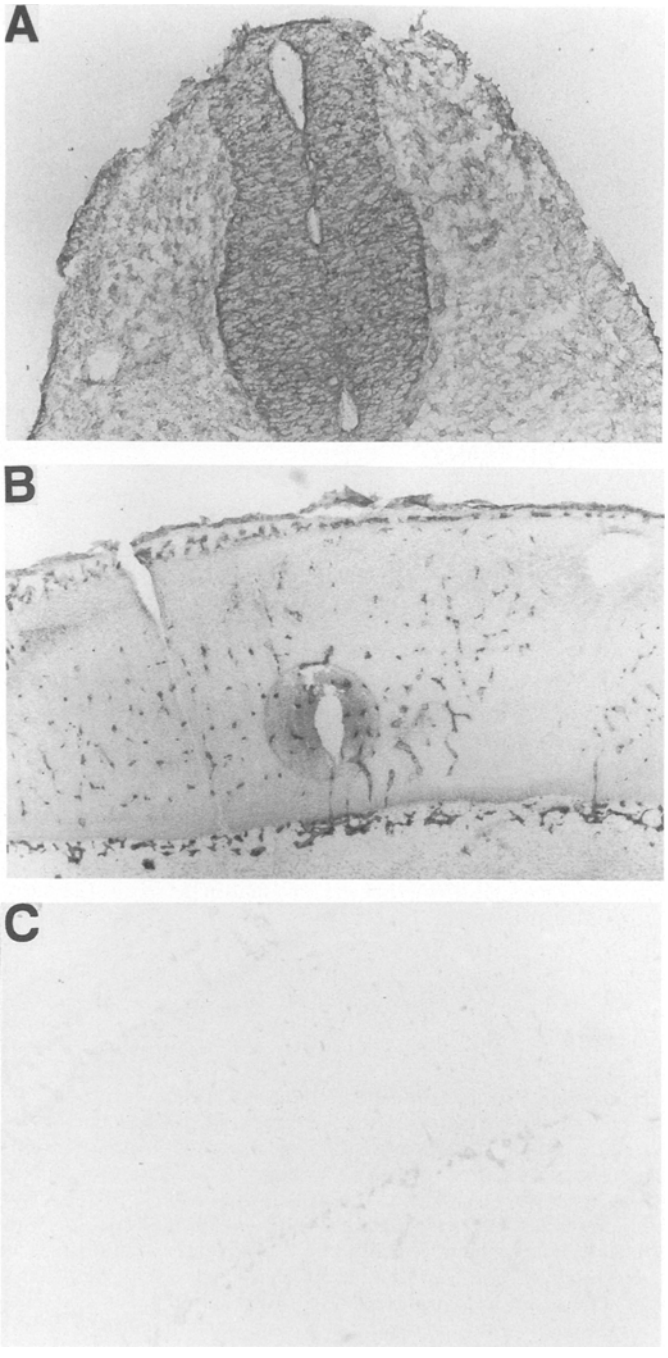


Fig. 5 A–C. Immunohistochemical staining of GLUT-1 protein of neural tube of the embryo on day 11 (**A**) and day 13 (**B**) (original magnification A; × 100, B; × 50). Transverse section of the embryo on day 11 and day 13 were treated with rabbit GLUT-1 antiserum. (**C**) represents a transverse section of embryo on day 13 in treatment with normal (non-immune) rabbit serum (1:100) (original magnification × 50). GLUT-1 protein immunoreactivity was observed in the neuroepithelium on day 11 and the microvessels on day 13

tion in PBS) was performed. The primary antibody incubation was then followed by the incubation with peroxidase-labelled protein A (Sigma, St. Louis, MO., USA) (1:11 dilution in PBS) secondary antibody for 45 min and with 3,3′-1-diaminobenzidine-0.03% H_2O_2 for 5 min. For control, normal rabbit serum (1:100) and PBS buffer alone were used with omission of the GLUT-1 antiserum.

Statistical analysis

All data are presented as mean ± SEM. Group comparisons were performed using Student's t-test for unpaired groups. Intergroup differences for comparison of morphologic lesions in embryos were assessed by chi-square analysis.

Results

Northern blot analysis revealed the presence of both GLUT-1 and GLUT-3 mRNA in the embryo and yolk sac during the period of organogenesis (Fig. 1, Fig. 2). GLUT-1 mRNA in the embryo and yolk sac hybridized to the GLUT-1 cDNA probe was about 2.8 kb in size, similar to that reported previously [1, 2] (Fig. 1). GLUT-1 mRNA expression was most abundant in the embryo on day 10 of gestation and decreased as the gestation progressed, while persistently abundant expression of GLUT-1 mRNA was observed in the yolk sac during day 10–13 of gestation (Fig. 1). Mouse GLUT-3 cDNA was probe-hybridized to a single transcript of about 4 kb in the embryo and yolk sac (Fig. 2), indicating no discernible cross-hybridization between GLUT-1 and GLUT-3 mRNA. GLUT-3 mRNA was expressed in the embryo and yolk sac during the early stage of organogenesis (Fig. 2).

The GLUT-4 cDNA probe did not hybridize to RNA from the embryo and yolk sac (data not shown). The autoradiogram of the Western blot of GLUT-1 protein is shown in Figure 3. The size of GLUT-1 protein bound to GLUT-1 antibody was approximately 45 kDa. Similar to the level of GLUT-1 mRNA expression, GLUT-1 protein level was the highest in the embryo on day 10, followed by gradual decline as the embryo developed. In the yolk sac high levels of GLUT-1 protein were observed during day 11–13 of gestation.

Immunohistochemical staining of GLUT-1 protein in the embryo which by Western blot showed their highest level on day 10 was found principally in the neuroepithelium and also in the heart (Fig. 4 A). However, immunoreactivity of GLUT-1 protein in the embryo, which by Western blot showed lower expression on day 13, was found in the microvessels surrounding neural tube, but much decreased in the neuroepithelial cells (Fig. 5). Further experiments were performed to examine whether the glucose transporter genes (GLUT-1 and GLUT-3 mRNA) and protein (GLUT-1) in the embryo and yolk sac, all of which were predominantly expressed during day 9–10, could be regulated in culture with high glucose medium. The expression of GLUT-1 and GLUT-3 mRNA in the embryo and yolk sac was not down-regulated in culture with high glucose media containing an additional 33.3 mmol/l and 66.6 mmol/l glucose for 24 h (Fig. 6, Fig. 7) and 48 h (data not shown) compared to control. The levels of GLUT-1 protein as well as glucose transporter genes were not suppressed by culturing in high glucose media for 24 h (Fig. 8). However, GLUT-1 protein significantly decreased in the embryo and yolk sac, when cultured in media containing an additional 33.3 mmol/l and 66.6 mmol/l glucose for 48 h (Fig. 9). Immunohistochemical staining of

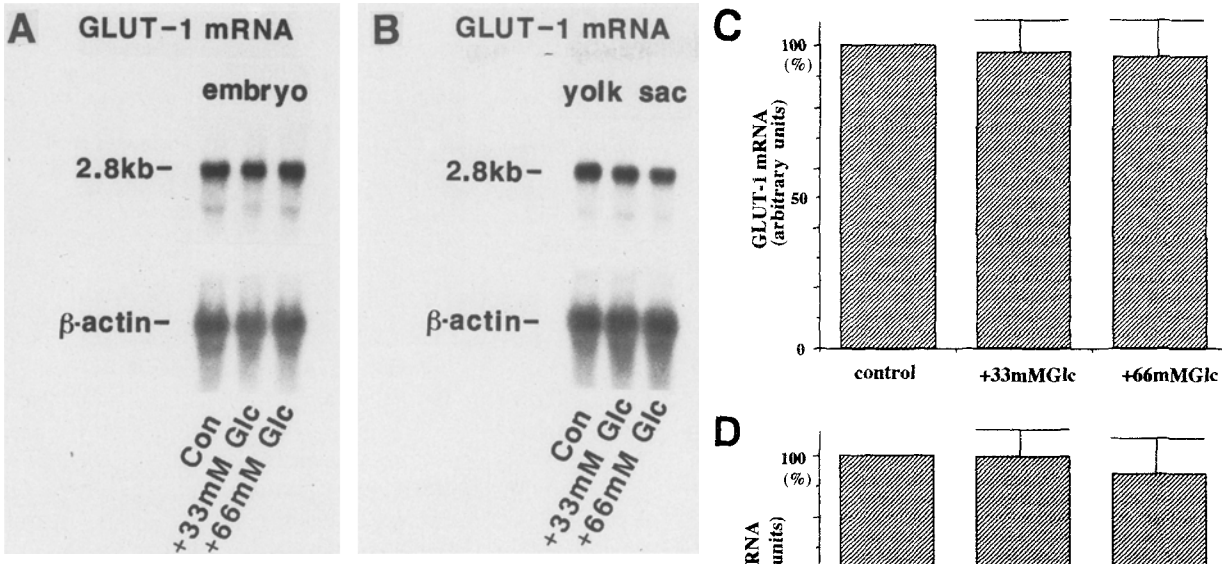


Fig. 6 A–D. Representative Northern blot (**A**, **B**) and quantitation (**C**, **D**) of GLUT-1 mRNA from the embryo and yolk sac cultured in control or high glucose medium for 24 h. Rat conceptuses on day 9 were cultured for 24 h in control (Con) and medium supplemented with 33.3 mmol/l glucose (+ 33 mM Glc) and 66.6 mmol/l glucose (+ 66 mM Glc). Northern blot analysis was performed in the separated embryo and yolk sac as described in Figure 1. The optical density values were expressed in arbitrary units of absorbance relative to the value showing the maximal level of GLUT-1 mRNA for each tissue. Each bar is mean ± SEM for three independent determinations

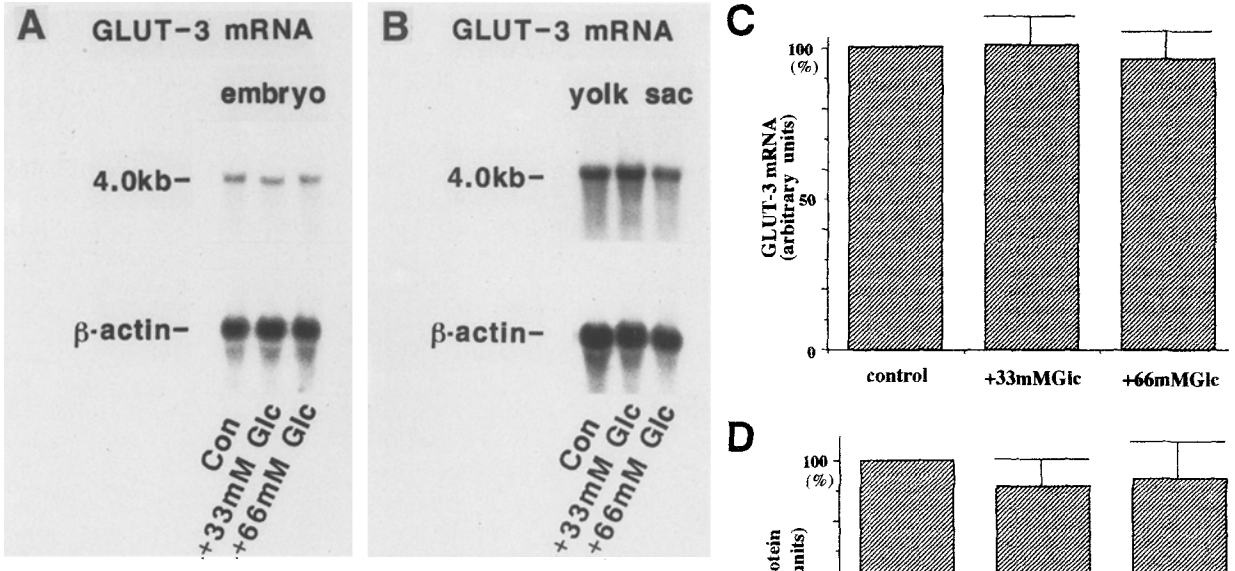


Fig. 7 A–D. Representative Northern blot (**A**, **B**) and quantitation (**C**, **D**) of GLUT-3 mRNA from the embryo and yolk sac cultured in control and high glucose medium for 24 h. Rat conceptuses were cultured for 24 h in control (Con) and medium supplemented with 33.3 mmol/l glucose (+ 33 mM Glc) and 66.6 mmol/l glucose (+ 66 mM Glc). Northern blot analysis was performed in the separated embryo and yolk sac as described in Figure 1. The optical density values were expressed in arbitrary units of absorbance relative to the value of the culture condition showing the maximal level of GLUT-3 mRNA. Each bar is mean ± SEM for three independent determinations

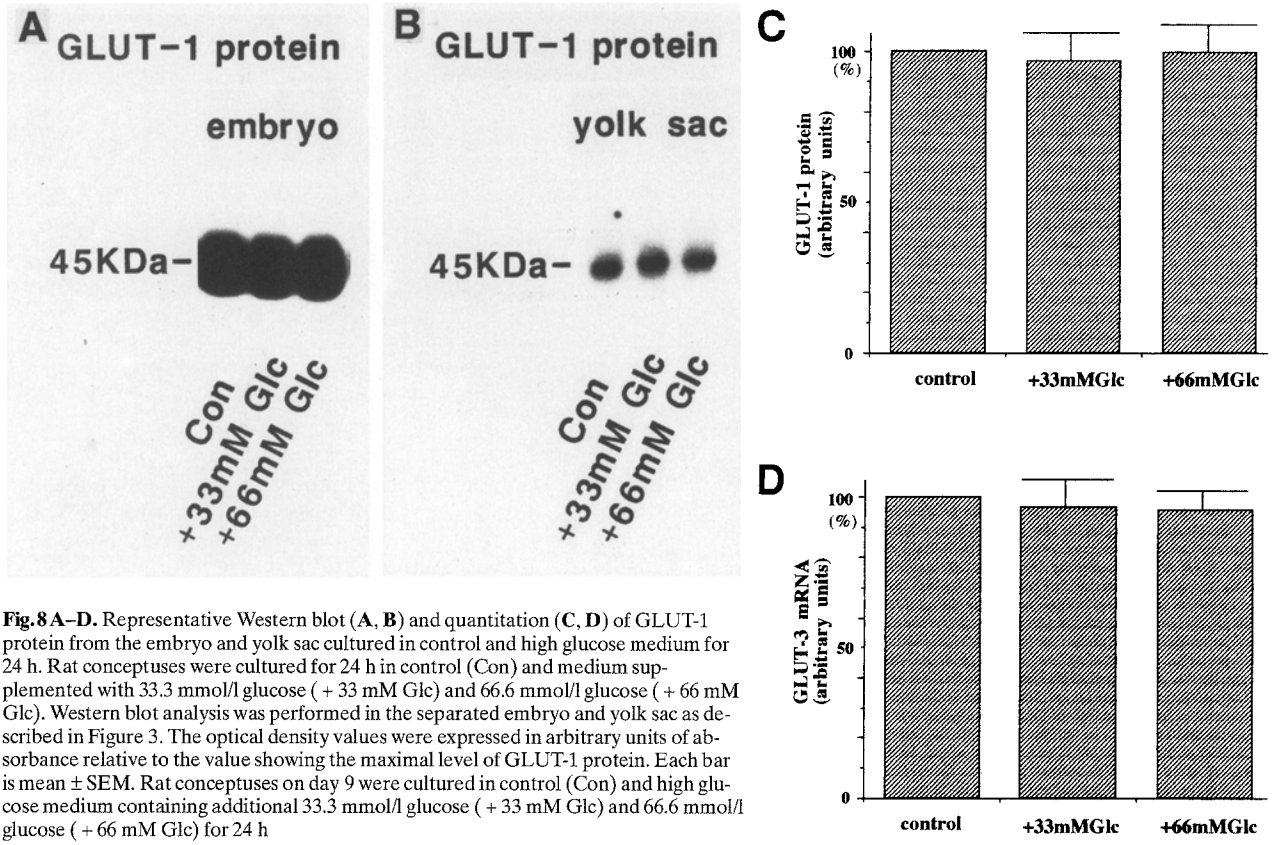


Fig. 8 A–D. Representative Western blot (**A**, **B**) and quantitation (**C**, **D**) of GLUT-1 protein from the embryo and yolk sac cultured in control and high glucose medium for 24 h. Rat conceptuses were cultured for 24 h in control (Con) and medium supplemented with 33.3 mmol/l glucose (+ 33 mM Glc) and 66.6 mmol/l glucose (+ 66 mM Glc). Western blot analysis was performed in the separated embryo and yolk sac as described in Figure 3. The optical density values were expressed in arbitrary units of absorbance relative to the value showing the maximal level of GLUT-1 protein. Each bar is mean ± SEM. Rat conceptuses on day 9 were cultured in control (Con) and high glucose medium containing additional 33.3 mmol/l glucose (+ 33 mM Glc) and 66.6 mmol/l glucose (+ 66 mM Glc) for 24 h

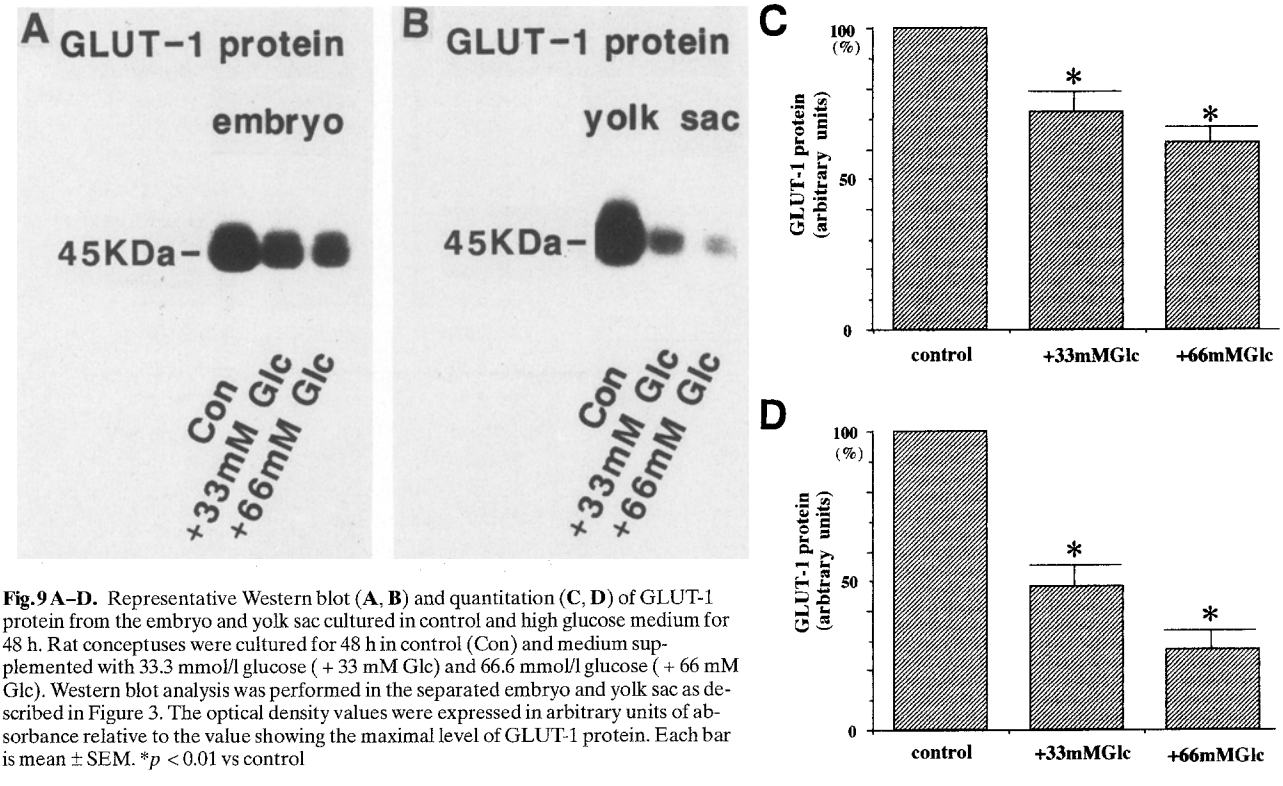


Fig. 9 A–D. Representative Western blot (**A**, **B**) and quantitation (**C**, **D**) of GLUT-1 protein from the embryo and yolk sac cultured in control and high glucose medium for 48 h. Rat conceptuses were cultured for 48 h in control (Con) and medium supplemented with 33.3 mmol/l glucose (+ 33 mM Glc) and 66.6 mmol/l glucose (+ 66 mM Glc). Western blot analysis was performed in the separated embryo and yolk sac as described in Figure 3. The optical density values were expressed in arbitrary units of absorbance relative to the value showing the maximal level of GLUT-1 protein. Each bar is mean ± SEM. $*p < 0.01$ vs control

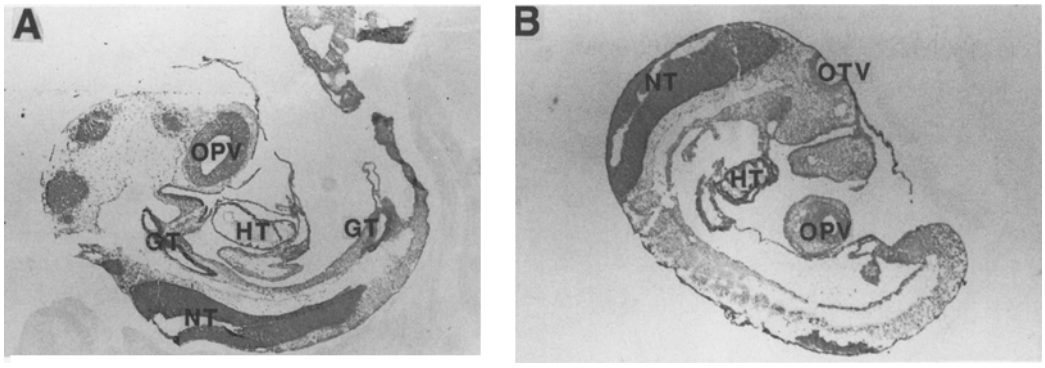


Fig. 10 A, B. Sagittal sections of immunohistochemical staining of GLUT-1 protein in the embryo (on day 10) cultured in control (**A**) and high glucose medium (**B**). Rat conceptuses on day 9 were cultured in control medium and medium supplemented with 33.3 mmol/l glucose (**B**) for 24 h. Immunohistochemistry was performed as described in Figure 4. GLUT-1 protein immunoreactivity was more prominent in the neural tube (NT) and also in the optic vesicle (OPV), the otic vesicle (OTV), the gut (GT) and the heart (HT)

GLUT-1 protein in embryos cultured in control and high glucose medium for 24 h is shown in Figure 10. GLUT-1 immunoreactivity in embryos cultured in high glucose medium was prominent and not down-regulated, when compared to control. Also, the tissue distribution of GLUT-1 protein was not changed during the high glucose culture (Fig. 10).

Control embryos explanted at the early head fold stage (day 9 of gestation) which developed normally during the 48-h culture period were indistinguishable from those observed in vivo. These embryos showed a clearly delineated brain, complete closure of the neural tube, well-formed otic and optic vesicles and normal heart development of an S-shaped tubular heart and complete axial rotation with a ventrally concave C-shaped curvature. In contrast, those embryos cultured in high glucose for 48 h exhibited an increased frequency of the neural defects associated with growth retardation (Table 1).

Discussion

In the present study, we demonstrated the predominant expression of high affinity glucose transporters (GLUT-1 and GLUT-3) mRNA in embryo and yolk sac during the early period of organogenesis. The level of GLUT-1 protein in the embryo increased in parallel with the expression of GLUT-1 mRNA, showing the highest level on day 10. Immunohistochemical staining of GLUT-1 protein showed high expression in the neuroepithelium, which did not decrease with 24-h high glucose culture (day 9–10) during the critical period of neural tube formation and was only down-regulated (day 9–11) by prolonged (48 h) high glucose exposure. The embryos cultured in high glucose showed an increased frequency of neural tube defects.

The conceptus during this period consisted of the embryo and extraembryonic membrane such as allantois, amnion and yolk sac. In the circulatory system, the yolk sac circulation begins to be established on day 10 and is fully developed by day 11. Since the chorioallantoic circulation is not formed until day 11, the embryo at this stage takes the nutrients (such as glucose and amino acids) or oxygen via direct diffusion from the yolk sac and then via the yolk sac circulation. The major energy production system of conceptus during early organogenesis is characterized by anaerobic glycolysis; a very high rate of glucose utilization and conversion to lactic acid has been demonstrated [11, 12]. Our finding that the high affinity glucose transporter genes (GLUT 1 and GLUT 3) and protein (GLUT 1) were expressed predominantly in the embryo and yolk sac during early organogenesis is consistent with the high requirement of glucose under glycolytic dependence. In a short-term incubation study of the separated embryo and extraembryonic membrane (including yolk sac), the finding that the rate of glycolysis in the yolk sac is much higher than that in the separate embryo (S, Akazawa and B. E. Metzger, unpublished data) was also consistent with higher expression of the high affinity glucose transporter genes and protein in the yolk sac than embryo.

During days 9 to 11 of gestation the major part of neural tube formation of the rat embryo occurs; the elevation and subsequent apposition of the neural fold and closure of the neural tube are completed by closing the posterior neuropore on day 11.3. High expression of GLUT-1 mRNA and GLUT-1 protein in the embryo was observed during day 9–11 during the major part of neural tube formation. Strong immunohistochemical staining of GLUT-1 protein of the embryo on day 10 was observed in neuroepithelium surrounding the neural tube. The level of GLUT-1 mRNA and protein decreased as gestational age progressed, especially after day 12, when complete closure of the neural tube takes place. Immunoreactivity of GLUT-1 protein of embryo decreased considerably in the neuroepithelium after day 12 and was then found in the microvessels surrounding the neural tube. GLUT-1 and GLUT-3 mRNA have been reported to be expressed in most adult tissues and to be abundant in the brain [46–49], although the expression of the GLUT-3 gene is restricted to the brain of rat and mouse. GLUT 1 and GLUT 3 are expressed differently in the brain; GLUT 1,

Table 1. Effects on growth and development of embryo after exposure to high glucose serum

Normal culture medium supplemented with	*n*	Crown rump (mm)	Somite (*n*)	Major lesions (%)	Minor lesions (%)
0	49	3.35 ± 0.04	28.3 ± 0.1	0	6.1
33.3 mmol/l D-glucose	43	3.16 ± 0.07[a]	26.3 ± 0.7[b]	4.7	30.2[b]
66.6 mmol/l D-glucose	35	2.85 ± 0.09[b]	25.7 ± 0.6[b]	22.8[b]	45.7[b]

Number of embryos examined indicated by *n*. Major lesions are neural lesions consisting of defects of neural tube closure, adhesion of posterior and anterior neural tube, and brain malformations. Minor lesions are defects in axial rotation or lesions involving the optic and otic vesicles, heart, pericardial cavity or skeletal system.
[a] $p < 0.05$, [b] $p < 0.01$ vs embryos cultured in control media

in the endothelial cells of the blood-brain barrier [50, 51] and GLUT 3, in the brain parenchymal cells [7]. GLUT-1 protein immunoreactivity has been reported to be distributed in the brain vascular endothelial cells or the fetus and neonate in rat and rabbit [46–48]. Altogether GLUT-1 protein is initially distributed in the neuroepithelium during the period of neural tube formation (day 9–11) and then in the microvessel after the closure of neural tube (day 12). Smith and Gridley [52] recently reported that expression of GLUT-1 and GLUT-3 mRNA in early postimplantation mouse embryo by in situ hybridization. They reported that both glucose transporters were expressed more strongly in extraembryonic tissue (including yolk sac and amnion) than in the embryo. Expression of GLUT-1 mRNA was widespread in the embryo (spinal cord, eye), while GLUT-3 mRNA expression was confined to (non-neural) surface ectoderm of embryo [52]; these are similar to our results. In the next experiment, we investigated the regulation of the high affinity glucose transporter genes (GLUT 1 and GLUT 3) and the protein (GLUT 1) in the embryo and yolk sac in high glucose culture. We, and others [22, 24, 26] have reported that the addition of glucose to culture media caused an excess accumulation of intracellular glucose which resulted in increased sorbitol accumulation and reduced myo-inositol content in the embryos in a dose-dependent fashion. The decrease of myo-inositol content may be attributable to the competitive inhibition by glucose of myo-inositol transport into the developing conceptus [26]. Myo-inositol supplementation to the culture media resulted in a significantly decreased incidence of malformation with complete restoration of myo-inositol contents in the embryos [24, 25], while aldose reductase inhibitor did not modify the incidence of malformations [22, 24]. Hyperglycaemia-induced teratogenesis has been suggested to be mediated by myo-inositol depletion in the embryos at a critical stage of organogenesis, which may be caused by competition with ambient glucose for uptake. Eriksson et al. [27] reported that the addition of free oxygen radical scavenging enzymes to high glucose culture media could protect hyperglycaemia-induced embryonic malformations, indicating that hyperglycaemia-induced embryopathy might be caused by generation of free oxygen radicals. In any case, an increased flux of glucose into the cell by hyperglycaemia may cause the excess formation of free oxygen radicals and myo-inositol depletion by competing with ambient glucose, leading to abnormal embryonic development.

It has been demonstrated that the ambient glucose concentration regulates the glucose transport activity in a variety of cell types. Glucose deprivation resulted in a specific elevation of glucose transport activity [30–34], which was associated with an increase in the number of glucose transporters. On a molecular basis, it was reported that the increase of glucose transport activity is associated with increased levels of GLUT-1 mRNA or protein in rat brain glial cells [33], primary cultured adipocytes [35], 3T3 adipocyte [36] and L6 muscle cells [34, 37]. Contrary to hypoglycaemia, hyperglycaemia has been reported to drecease glucose transport activity in many cell types [35, 39–43], indicating down-regulation of the glucose transport system. The decreased activity is associated with a decreased number of glucose transporters or reduced levels of the glucose transporter gene and/or protein in the primary cultured adipocytes [35], microvessels of blood brain barrier [39], rat adipocytes [41], 3T3-L1 fibroblasts [42] and hamster-derived pancreatic beta-cell line (HIT) [43].

In our experiments, the high expression of GLUT-1 protein was not down-regulated during the 24-h high glucose culture (day 9–10) but down-regulated only during the prolonged (48 h) exposure to high glucose (day 9–11). The levels of GLUT-1 and GLUT-3 mRNA were not affected by the 24-h high glucose culture. The first 24 h of culture (day 9–10) in high glucose medium have been shown to be the critical period during which embryonic malformations such as neural tube defects can be induced [17–20]. The lack of down-regulation and continuous, high expression of the high affinity glucose transporters during this critical period may permit an increased flux of glucose into the differentiating neuroepithelial cells, which may induce the generation of free oxygen radicals or myo-inositol depletion.

In summary, the abundant expression of high affinity glucose transporters in the rat embryo during the major period of neural tube formation was demonstrated. This increased expression of glucose transporters was not down-regulated during the critical developing period in the presence of high glucose, which may permit increased glucose flux into the neuroepithelial cells. This process may be one of those involved in neural tube defects induced by hyperglycaemia.

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